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Bionomics and host plants of the invasive *Cydia interscindana* (Möschler, 1866) (Lepidoptera, Tortricidae), an emerging pest in the Carpathian Lowlands

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Abstract. *Cydia interscindana* (Möschler, 1866) has spread through several European countries in the past few years, becoming an invasive pest of ornamental trees. It was collected in Hungary for the first time in a pheromone trap set for *Cydia pomonella* (Linnaeus, 1758) in 2014. Here we discuss its recent distribution in Hungary based on intensive sampling between 2018 and 2020, which showed the dispersal of the pest by humans. Two formerly unknown host plants are also recorded. The damage caused by the larvae, the external morphology of the adult male, larva, pupa (described for the first time) and pupal exuviae are presented. We also analyse DNA barcodes, identifying this pest for the first time via DNA sequencing of immature stages.

Introduction

Cydia interscindana is native in the Mediterranean region, where it was described by Möschler in 1866 from Andalusia. It is distributed in Mediterranean countries including Portugal (Corley 2004), Spain (Férriz et al. 2006), France (Lévêque et al. 2017) and Italy (Minelli 1995). Later the species was recorded in the British Isles (Knill-Jones 2020), Belgium (De Prins 2016), Switzerland (Swisslepteam 2010), Slovakia (Pastorális et al. 2018) and Russia (Caucasus; Schurov et al. 2017). In Hungary, *Cydia interscindana* adults were caught by a sticky delta pheromone trap (CSALOMON RAG type) for *Cydia pomonella* (Linnaeus 1758) in 2014 during a study on swarming dynamics of the latter pest in Budapest. This provided the first record of the species in the Carpathian basin (Szabóky 2014; Takács and Szabóky 2015).

In the Mediterranean region larvae feed on *Juniperus oxycedrus* (L.) (Miller 1990). In Belgium the larva was recorded on *Juniperus* spp. (Meert et al. 2019). *J. oxycedrus* is not native in Hungary, but *Cupressus* × *leylandii* A.B. Jacks. & Dallim 1926, *Platycladus orientalis* (L.) Franco 1949 and *Chamaecyparis lawsoniana* (A. Murray bis) Parl. 1864 are popular evergreens used as ornamental trees both in parks and gardens. In Hungary several pests of these plants have been recorded, all probably introduced with imported plants; in the literature, 11 Lepidoptera, nine Coleoptera and six Hemiptera species have been mentioned already (Csóka and Kovács 1999; Maráczi 2013; Bozsik et al. 2016; Schurov et al. 2017). However, until the end of the 2000s, only Scolytidae (Coleoptera) species caused serious damage (Bozsik and Szőcs 2017). In 2012, an outbreak of the formerly detected (Muskovits 2001) *Lamprodila festiva* (Linnaeus 1767) (Buprestidae) took place in Budapest (Németh 2012) causing serious damage on *Platycladus orientalis* and several ornamental gymnosperm species. This outbreak was certainly caused by introduced specimens, that had arrived with trees from the Mediterranean region where this beetle is a well-known pest (Merkl 2016), whose abundance in Hungary increases due to climatic change (Csóka et al. 2018). Based on the available data, in Hungary this beetle pest has also been blamed for all the damage caused on *Cupressus*, *Platycladus* and *Chamaecyparis* trees and management has been carried out only against them.

In 2018, a larva of *L. festiva*, an unidentified caterpillar and a freshly emerged specimen of *Cydia interscindana* were collected simultaneously from a Leyland cypress in Székesfehérvár (Central Hungary). In that year, similar Lepidoptera larvae were found in three neighbouring villages: Velence, Sukoró and Pákozd. To identify the sampled caterpillar, DNA analysis was undertaken. Additionally, in 2019–2020 a country-wide investigation was carried out to map the distribution and abundance of *C. interscindana* and gather data on bionomics of this pest in the Carpathian basin.

Materials and methods

Samplings

On 25th September 2018 *Cupressus* × *leylandii* trees were examined in the suburban region of Székesfehérvár from where a freshly emerged specimen of *Cydia interscindana* had been received from a horticulturist. Larvae of *L. festiva* (Fig. 2) and an unidentified Lepidoptera species (Fig. 3) were collected from under tree bark. The caterpillar soon died, but it was preserved for genetic analysis. In that year similar Lepidoptera larvae were found in three neighbouring villages (Velence, Sukoró, Pákozd).

Table 1. Sampling sites studied during the investigation on the distribution of *C. interscindana* in Hungary between 2014 and 2020 with habitat type and available potential host plants. Cley = *Cupressus* × *leylandii*, Pori = *Platycladus orientalis*, Claw = *Chamaecyparis lawsoniana*.

Site	Coordinates	Habitat and available host plants	Sampling period
Székesfehérvár	47.20560°N, 18.43125°E	suburban site, house garden; Cley	2018, 2020
Zalaegerszeg	46.83398°N, 16.85150°E	suburban site, house garden; Cley	2019
Békéscsaba	46.67891°N, 21.07516°E	suburban site, house garden; Cley	2019, 2020
Kápolnásnyék	47.23337°N, 18.66671°E	suburban site, house garden; Cley	2019, 2020
Velence	47.24280°N, 18.64627°E	suburban site, house garden; Cley	2020
Sukoró	47.24367°N, 18.61935°E	suburban site, house garden; Cley	2020
Pákozd	47.23182°N, 18.56900°E	suburban site, house garden; Cley	2020
Gárdony	47.19067°N, 18.60952°E	suburban site, house garden; Cley	2020
Budapest	47.50044°N, 19.28462°E	suburban site, house garden; Cley	2018, 2019, 2020
Debrecen 1	47.59333°N, 21.56333°E	suburban site, house garden; Cley	2020
Debrecen 2	47.59273°N, 21.56222°E	suburban site, house garden; Cley	2020
Debrecen 3	47.53275°N, 21.51805°E	suburban site, house garden; Cley	2020
Nyíregyháza	47.97244°N, 21.71108°E	botanic garden, Cley, Pori, Claw	2020
Derecske	47.36039°N, 21.56364°E	suburban site, house garden; Cley	2020
Bernecebaráti	48.03913°N, 18.91415°E	suburban site, house garden; Cley	2020
Horpács	47.99633°N, 19.12929°E	suburban site, house garden; Cley	2020
Nagyatád 1	46.22666°N, 17.39333°E	suburban site, house garden; Cley	2020
Nagyatád 2	46.23000°N, 17.36333°E	suburban site, house garden; Cley	2020
Kaposvár	46.36833°N, 17.77000°E	suburban site, house garden; Cley	2020
Bugac	46.65333°N, 19.60000°E	suburban site, house garden; Cley	2020
Berettyóújfalu	47.22500°N, 21.53500°E	suburban site, house garden; Cley	2020

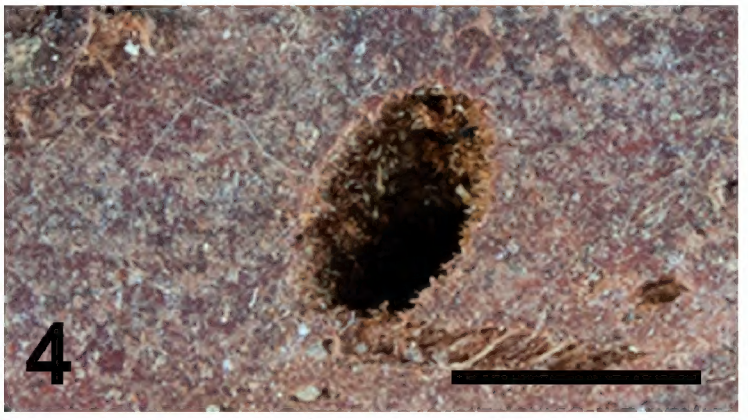
In order to collect actual data on the distribution of *C. interscindana*, an investigation was launched in 2019, when CSALOMON-type *C. pomonella* pheromone traps were used at three sites: in Zalaegerszeg, Békéscsaba and Kápolnásnyék. In 2020 this survey was extended country-wide to a total of 18 localities: Békéscsaba, Berettyóújfalu, Bernecebaráti, Budapest, Bugac, Debrecen, Derecske, Gárdony, Horpács, Kápolnásnyék, Kaposvár, Nagyatád, Nyíregyháza, Pákozd, Sukoró, Székesfehérvár, Velence, Zalaegerszeg and with 21 sampling sites in total (Fig. 1 and Table 1). In 2020 the same type of pheromone trap was used. Two traps were used in each site on or close to potential host plants of the pest. During the studies, traps worked from mid-May to the end of September. Traps were generally checked once a week except in Budapest where they were checked yearly. Baits were changed after four weeks, whereas sticky sheets were changed if it was necessary.

Identification of sampled adults (Fig. 6) was made based on the description of Razowski (2003). Where traps caught enough *C. interscindana*, data were used to characterise population dynamics and life cycle of the species.

In 2019 and 2021 further damages were studied and larvae were collected in Budapest and Velence. Intact specimens and larval damage were documented with photographs made with a Canon 450 D camera, applied to a Carl Zeiss Stemi-2000 binocular stereomicroscope.

DNA extraction

Three specimens were selected for molecular analyses (Table 2). Specimens were killed and stored in 70% alcohol. The genomic DNA was isolated from two legs of adult specimens or from a piece of the larval body. DNA was isolated with Quick-DNATM Tissue/Insect Miniprep Kit (Zymo Research) according to the recommended protocol of the manufacturer.



Figures 1–6. Damage and developmental stages of pests in Cupressaceae. **1.** Larva of *Lamprodila festiva*; **2.** Damage caused by the larva of *Cydia interscindana* in the phloem; **3.** Tunnel of the larva of *Lamprodila festiva*; **4.** Exit hole of *C. interscindana*; **5.** Fully grown larva of *C. interscindana*; **6.** Adult of *C. interscindana*. Scale bars: 1 mm (Figs **1, 3, 5, 6**); 10 mm (Fig. **2**); 5 mm (Fig. **4**). Photos were taken in Velence on 15.05. 2019 (**5**), 15.09.2019. (**2**) and 10.01.2021 (**1, 3, 4**) and in Budapest on 18.06.2016 (**6**) by Attila Takács.

Table 2. Data for *Cydia* specimens collected for molecular analyses from three Hungarian sampling sites. Samples were taken by A. Takács.

Species	Host plant	Locality	GPS: N, GPS: E	Date of collection	NCBI GenBank code
C. interscindana larva	*Cupressus* × *leylandii*	Székesfehérvár	47.20560°N, 18.43125°E	25.09.2018.	MW580708
C. interscindana adult	*Cupressus* × *leylandii*	Budapest	47.50044°N, 19.28462°E	30.08.2019.	MW580709
C. interscindana larva	*Chamaecyparis lawsoniana*	Velence	47.24280°N, 18.64627°E	05.01.2021.	MW591863

PCR amplification and sequencing

Amplification of the 658 bp DNA COI barcode region was performed with the primers LCO-1490 and HCO-2198 (Folmer et al. 1994). The PCR mixture contained 2 µl of template DNA, 7.5 µl of 2× HotStart Taq Plus Master Mix (HotStart Taq Plus Master Mix Kit, Qiagen), 0.5 µl

of each primer (10 µM) and double distilled water to a final volume of 15 µl. The amplification profile consisted of a preheating step of 5 min at 95 °C followed by 5 cycles of 95 °C for 30 seconds (s), 45 °C for 30 s and 72 °C for 1 min, and additional 35 cycles at 95 °C for 30 s, 51 °C for 1 min and 72 °C for 1 min and a final extension at 72 °C for 10 min. Reaction was performed in a PTC-100 DNA thermal cycler (MJ Research). Products of amplification were analysed in 2% agarose gel with addition of ethidium bromide and visualised under UV light. After visualization of the PCR-products on agarose gel, they were purified and sequenced bi-directionally (BaseClear B.V.). Specimens used in this study are listed in Table 1 along with GenBank accession numbers.

Sequence analysis and phylogenetic reconstruction

The forward and reverse sequences were assembled with Staden Package 2.0.0b9. Sequences were inspected and translated using the translate tool of ExPASy Bioinformatics Resource Portal (Artimo et al. 2012) to verify that they were free of stop codons.

For further analysis, we used only available sequences of Austrian *C. interscindana* specimen with other gymnosperm-feeding *Cydia* species: *Cydia cognatana* (Barrett, 1874), *C. colorana* Kearfott, 1907, *C. conicolana* (Heylaerts, 1874), *C. duplicana* (Zetterstedt, 1839), *C. indivisa* (Danilevsky, 1963), *C. illutana* (Herrich-Schäffer, 1851), *C. inopiosa* (Heinrich, 1926), *C. phyllisi* Miller, 1986, *C. strobilella* (Linnaeus, 1758). Multiple sequence alignment was carried out by ClustalW (Larkin et al. 2007), with default parameters for gene sequences of examined *Cydia* species and with outgroup taxa (*Ecdytolopha insiticiana* Zeller, 1875). The distance analysis within the examined taxa was obtained based on uncorrected p-distances using MEGA 7 (Kumar et al. 2016).

The most appropriate model of DNA sequence analysis was determined with MEGA7 under the Bayesian Information Criterion (BIC). The General Time Reversible model with discrete Gamma distribution (GTR+G) (Tavaré 1986) was selected for our phylogenetic reconstruction. Phylogenetic trees were calculated with Maximum Likelihood (ML) method as implemented in MEGA 7, starting from a random Neighbour Joining tree, with the default initial rearrangement settings. To obtain an estimate of the support for each node, a bootstrap analysis using 5000 replicates was performed. Bootstrap support is given on appropriate clades in the ML tree.

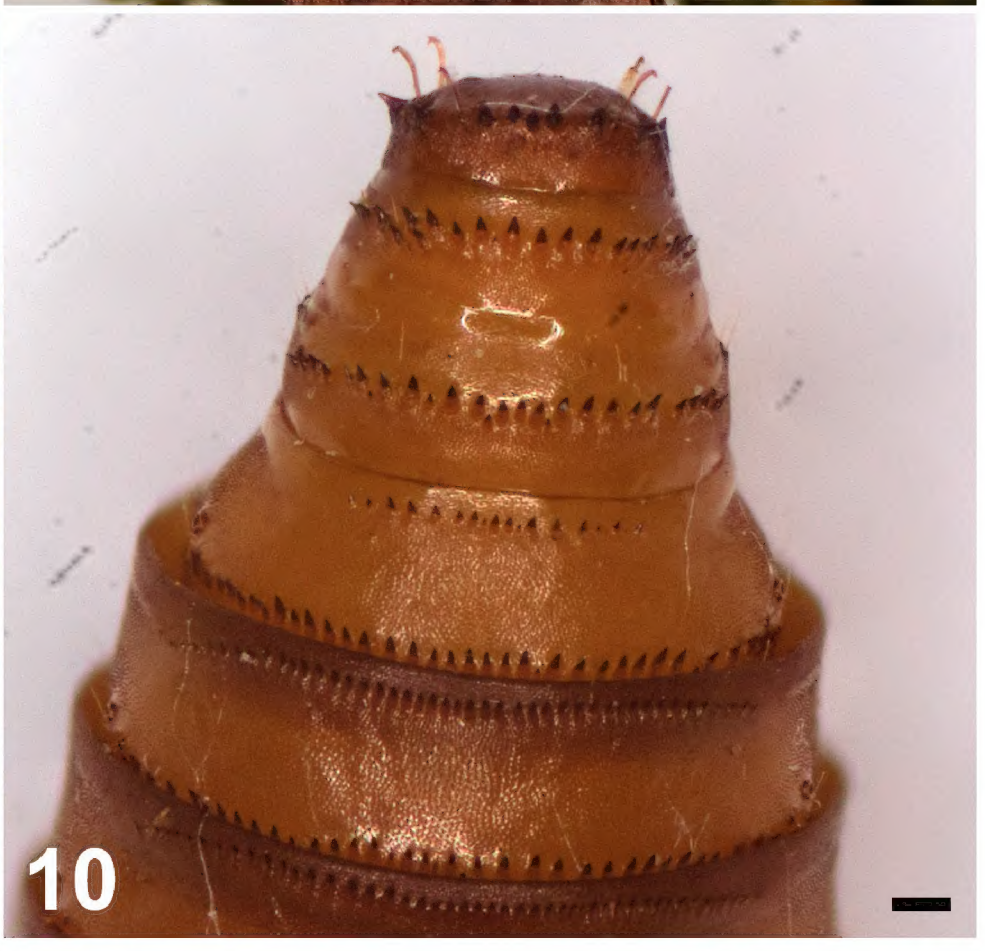
Results

Bionomic data of *Cydia interscindana*

Amongst preimaginal stages, ovum and morphology of larvae were not studied in detail, but damage and signs of occurrence that are important in the recognition of damage are described hereunder.

Mines

The mines of *C. interscindana* larvae can be found on the branches and trunk of the host plant. The mines are full of frass (Fig. 2) that differs conspicuously from that of the co-occurring *L. festiva* (Fig. 3). At the entrance of the tunnel, the beetle's reddish frass can be also found (Fig. 4). The mine reaches the phloem. The plant tissue around the mine becomes loose, slightly swollen (Fig. 8), somewhat sponge-like; this tissue is consumed by the larva. Perhaps it represents a case of gall-induction.



Figures 7–10. Pupa of *C. interscindana*. **7.** Exuviae, close view; **8.** Two exuviae on a *Chamaecyparis lawsoniana* tree; **9.** Pupa in wood, ventral view; **10.** Caudal end of pupa, dorsal view. Scale bars: 1 mm (Figs **7**, **9**); 10 mm (Fig. **8**); 0.1 mm (Fig. **10**). Photos were taken in Velence on 10.01.2021 (**7, 8**) and 10.05.2020 (**9, 10**) by Attila Takács.

Pupa

The pupa is light brown (Fig. 9). The caudal edge of each abdominal segment has a row of tiny, but strong teeth on the dorsal side, accompanied by an additional transverse row in the central third of the segment. The caudal end of the pupa is slightly rounded, nearly truncated, with three pairs of narrow and hooked cremastal setae (Fig. 10). The pupal stage lasts for 10–12 days.

Exuviae

The exuviae are brown, extruding by 2/3 from the wood (Figs 7, 8). They were found in Velence and Székesfehérvár.

Host plants and damage

In Hungary, *C. interscindana* was detected together with of *L. festiva*, on *Cupressus* × *leylandii*, *Platycladus orientalis* and *Chamaecyparis lawsoniana* (Cupressaceae). Pheromone traps were also used near one or two or even all of these host plants, and where it was possible, traps were placed on these trees.

Larvae consume the phloem (Fig. 2), which has the very important physiological role of transport and storage of nutrients. Due to the attacks the plants turn yellow, begin to fade and dry out and in cases of heavy infestation plants may even die.

All examined *Cupressus* × *leylandii*, *Platycladus orientalis*, and *Chamaecyparis lawsoniana* plants (n=52) were infected by *C. interscindana*, but *L. festiva* attacked only 30 plants. The number of *C. interscindana* larvae was highest in *Cupressus* × *leylandii*, while the least preferred host plant was *Platycladus orientalis* (Table 3).

Life cycle

The present study shows that *Cydia interscindana* develops two generations per year in Hungary and that these do not overlap. Adults of 1st generation are on the wing from mid-May to mid- or late June; those of the 2nd generation from mid-August until mid- or late September, depending on weather conditions (Fig. 11). Specimens overwinter as mature larvae and pupate in early spring.

Distribution in Hungary

The distribution of *Cydia interscindana* has been studied in 11 localities in Hungary between 2018 and 2019. In 2018 it was found in Székesfehérvár and damage that may have been caused by this pest was also detected in the three neighbouring localities of Velence, Sukoró and Pákozd. In 2019, traps collected *C. interscindana* in Békéscsaba and Kápolnásnyék, but not in Zalaegerszeg.

Table 3. Numbers of sampled larvae of *C. interscindana* and *L. festiva* in three Cupressaceae species from various localities.

	Velence	Sukoró	Pákozd	Gárdony	Kápolnás-nyék	Székes-fehérvár	Budapest	Total
				Cydia interscindana				
C. × *leylandii*	3	5	2	3	4	0	6	23
P. orientalis	4	2	2	1	0	1	2	12
C. lawsoniana	9	4	3	1	0	1	0	18
				Lamprodila festiva				
C. × *leylandii*	3	3	1	2	1	0	1	11
P. orientalis	2	4	1	1	0	1	2	11
C. lawsoniana	2	0	0	0	0	0	0	2

In 2020 *C. interscindana* specimens were caught by traps in Velence, Sukoró, Pákozd, Gárdony, Kápolnásnyék, Székesfehérvár, Békéscsaba, Debrecen and Budapest (Fig. 12).

Molecular analysis

The recovered length of the COI region of the mitochondrial DNA numbered 547 nucleotide positions in the final dataset. Based on the results of our analysis, the three Hungarian specimens had the same nucleotide sequence in this examined region. This was the reason for characterising the three Hungarian populations with a single sequence in our analysis. Relationships among examined species inferred from the analysed mitochondrial region are shown in Fig. 13.

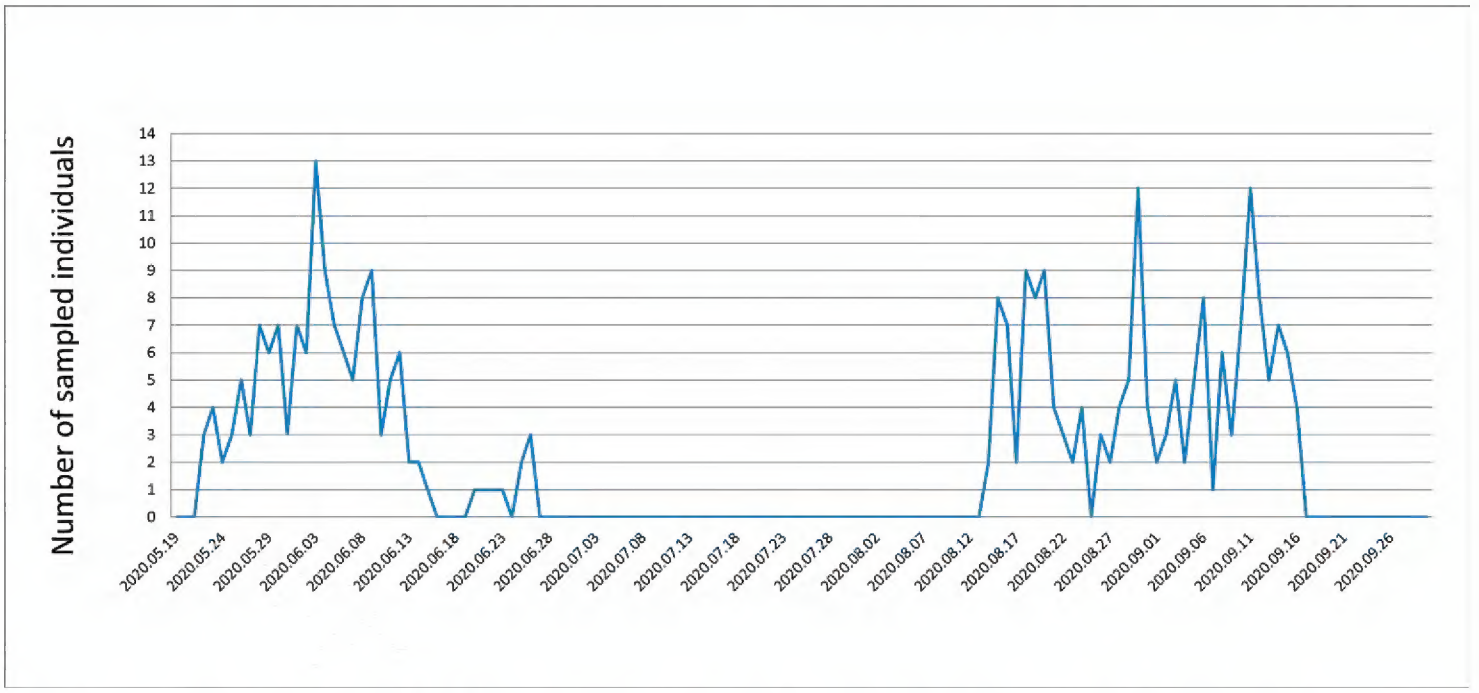


Figure 11. Temporal changes of 3-day moving average of abundance of *Cydia interscindana* based on *Cydia pomonella* sex pheromone trap catches in Budapest, 2020.

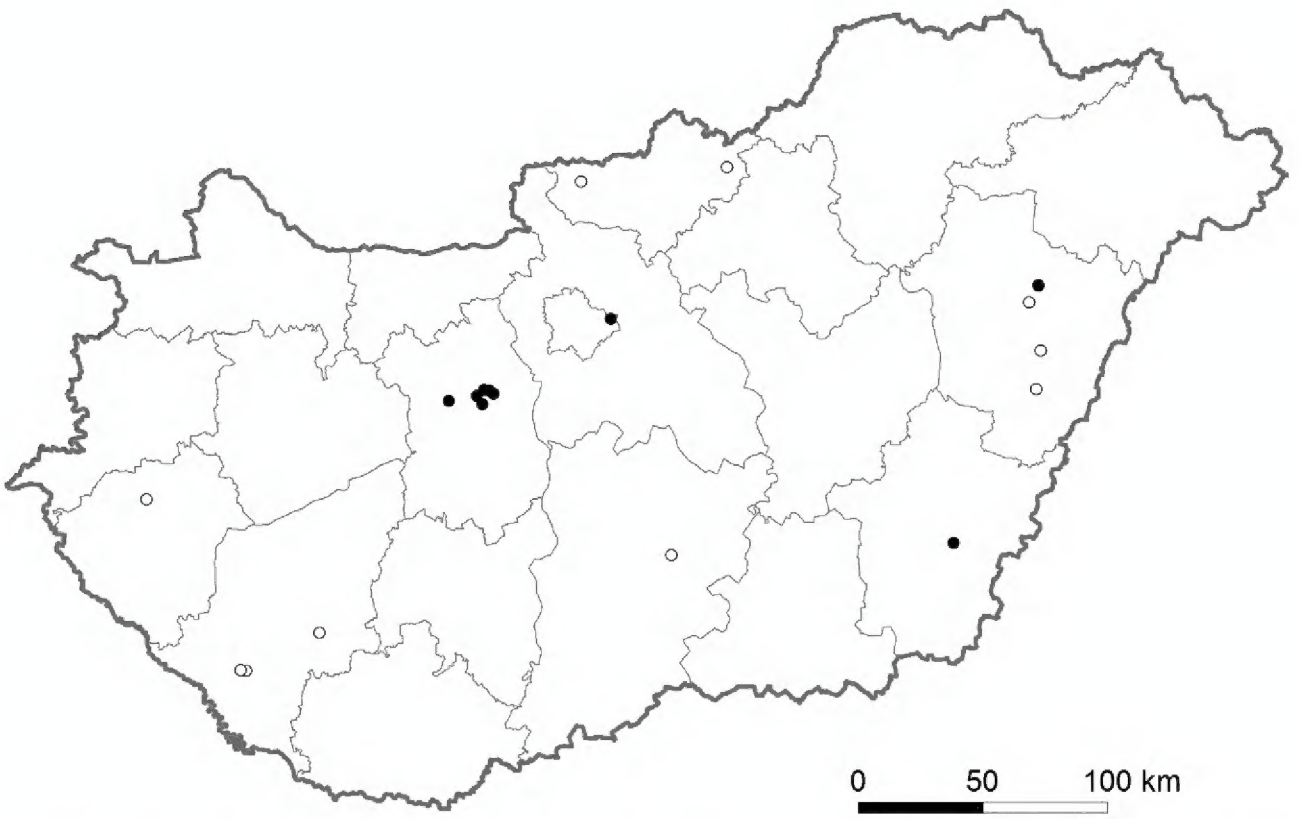


Figure 12. Known distribution of *Cydia interscindana* in Hungary. Black dots: the species is present; empty dots: the species was searched for but not found between 2018 and 2020. (Sampling sites are detailed in Table 1).

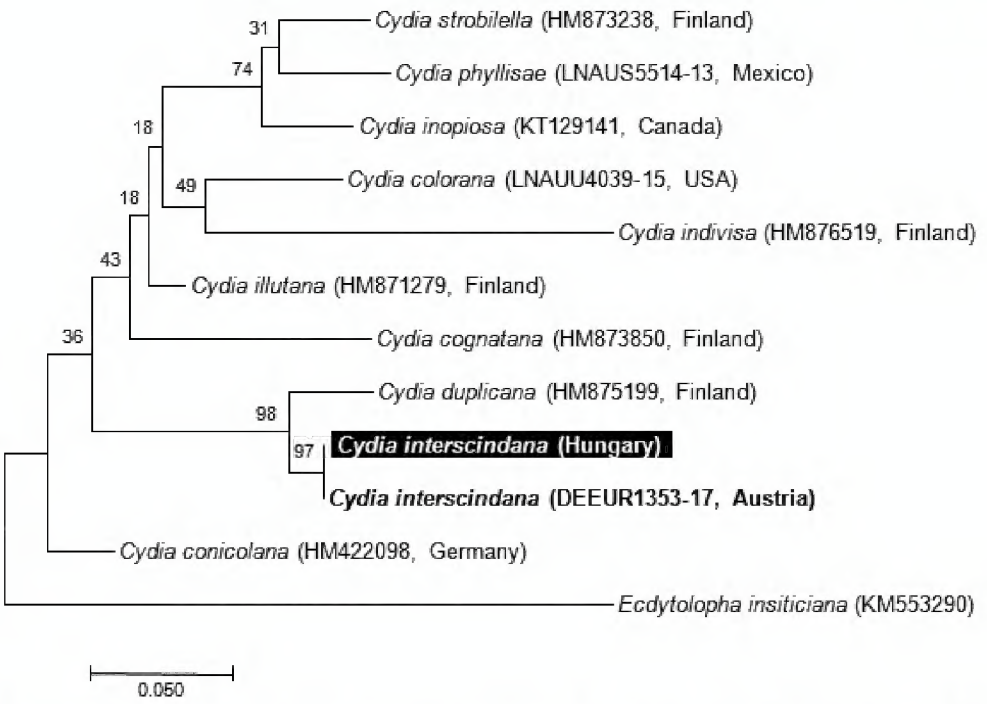


Figure 13. Relationships of examined *Cydia* species based on a ML analysis was inferred using COI-5P sequences (547 aligned nucleotides) under the GTR+G model. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (5000 replicates) is shown next to the branches. Species names and GenBank Accession or BOLD Sample/Process ID numbers are listed for each taxon. The scale bar represents the number of substitutions per site.

Within the "gymnosperm" group the three Hungarian *Cydia interscindana* populations shared an identical sequence with the Austrian specimen. These populations formed a common clade with the most closely related *Cydia* species (*C. duplicana*). The genetic distance between them was 3% (17 nucleotides). Since this value is very similar to typical species-level genetic differences (e.g. Huemer et al. 2014), we also assume that these two closely related species are not conspecific (bootstrap value was 98%).

Discussion

Based on specimens sampled in Székesfehérvár, Velence and Budapest, we have described morphology of pupa and their exuviae, damage and other signs of occurrence of *C. interscindana* were described. These immature specimens were identified, for the first time for this pest, based on molecular analysis and sequences uploaded to GenBank. Along with the formerly known morphology of adults (Möschler 1866; Razowski 2003; Knill-Jones 2020), all the characteristics necessary for recognition of the pest are provided.

In Hungary we record three new host plants for *C. interscindana*. The original host *Juniperus oxycedrus* is not native in Hungary, thus the moth feeds on ornamental *Cupressus* × *leylandii*, *Platycladus orientalis*, and *Chamaecyparis lawsoniana* (Cupressaceae) trees together with *L. festiva*. Our study shows that *L. festiva* is not the only pest on these three plants; *C. interscindana* also presents a significant problem especially on *Cupressus* × *leylandii* which was the most preferred among them. Other known host *Juniperus* spp. (e.g. *J. communis*: Miller 1990; Meert et al. 2019; Knill-Jones 2020) were not available at the present sites studied.

Based on pheromone trap catches, *C. interscindana* has two generations per year in Hungary, as it was formerly recorded by Takács and Szabóky (2015) and in other parts of its range, e.g., in France and the United Kingdom (Knill-Jones 2020).

In the three-year period between 2018 and 2020, *C. interscindana* was recorded in nine localities – most of them aggregated in the surroundings of Székesfehérvár. The two isolated populations in Debrecen and Békéscsaba shows that the pest spreads mainly by humans. Inadequately inspected plants provide a faster way for colonisation of new areas than does natural range expansion, since in case of introduction (or invasion) the propagule pressure is greater and the success of the colonisation is higher (Hoffmann and Courchamp 2016). The first discovered Hungarian population in Budapest has probably also been established by imported ornamental plants (Szabóky 2014; Takács and Szabóky 2015). Both in Hungary and elsewhere, such alien species may not encounter their predators, parasitoids, competitors or other limiting factors that are present in their original habitats (Gallien et al. 2012). Even if several parasitoid species live in their newly colonised area, they are unlikely to have been able to adapt to the introduced species during a short time. This is probably why these species become invasive and increase in population size, causing significant damages that are noticed by a broader range of people.

Although *C. interscindana* up to present has been found only in four counties of Hungary, it is probably much more widespread. In order to map the actual distribution, further investigations are needed. This moth, together with the beetle *L. festiva*, has caused significant damage in Hungary and their local populations can serve as "sources" for further spread. In the near future, a country-wide invasion of the pest is likely to take place and it can be slowed down only by strict examination of transported plant materials.

Our study emphasises that DNA barcodes are a useful tool for identification of the immature stages, not requiring larvae to be reared to adults for identification.

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